PATENT SPECIFICATION 980,282

NO DRAWINGS.

980,282

Date of Application and filing Complete Specification:
March 6, 1961. *No. 7970/61.*

Application made in United States of America (No. 17247) on March 24, 1960.

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Int. Cl.:—A 61 k.

COMPLETE SPECIFICATION.

Antibiotic Mastitis Composition.

ERRATA

PATENT SPECIFICATION NO. 980,282

AMENDMENT NO. 1

Page 1, line 17, *for* "amount" *read* "amounts"

Page 1, line 81/82, *for* "(Dichlorodifluoromethane," *read* "(Dichlorodiflouromethane)"

Page 1, line 82/ *for* "trichlorofluoroet..." *read* "trichloroflouroethane,"

Page 1, line 82/83, *for* "trichlorofluorometnane)." *read* "trichloroflourometnane)."

Page 2, line 1, *for* "lb." *read* "lbs"

Page 2, line 38, *for* "ethyl" *read* "othyl"

Page 2, line 56, *for* "preparted" *read* "prepared"

Page 2, line 97, *for* "fater" *read* "after"

Page 2, line 99, *for* "antiobiotic" *read* "antibiotic"

Page 3, line 21, *for* "strepococci" *read* "streptococci"

THE PATENT OFFICE
24th June, 1965

D 40726/4

position into the mammary gland through the relatively restricted teat opening is in no way analogous to such broadcast spraying for treatment of respiratory diseases of chickens and the like.

It is an object of the present invention to develop an improved mastitis treating composition.

Another object of the invention is to prepare an aqueous mastitis treating composi-

[*Price* 4*s.* 6*d.*]

the aerosol.

The preferred pressure generating propellant in the aerosol is nitrous oxide. Other nontoxic gaseous propellants which can be used include nitrogen, carbon dioxide and the compounds sold under the Registered Trade Mark "Freon" (Dichlorodifluoromethane, trichlorofluoroethane, and trichlorofluoromethane).

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COMPLETE SPECIFICATION.

Antibiotic Mastitis Composition.

We, PHILIPS ROXANE, INC., a Corporation organised under the laws of the State of Delaware, United States of America, of 2400 Frederick Avenue, Saint Joseph, Missouri, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to a composition for use in the treatment of mastitis.

Various mastitis treating compositions have been proposed in the past. Most of such formulations include penicillin. However, penicillin is frequently found in residual amount in the milk of cows treated for mastitis. Since many people are sensitive to minute amounts of penicillin the presence of the residual penicillin in the milk is undesirable.

While it has previously been proposed to prepare aerosol compositions for combatting respiratory diseases comprising solid streptomycin or dihydrostreptomycin and a nontoxic pressure generating propellant, see U.S. Patent 2,802,772, in such procedure, the aerosol is simply sprayed into the atmosphere. The use of liquid aerosols is stated by Elder as being ineffective for his purpose. It will be appreciated that the problem of infuring a mastitis treating composition into the mammary gland through the relatively restricted teat opening is in no way analogous to such broadcast spraying for treatment of respiratory diseases of chickens and the like.

It is an object of the present invention to develop an improved mastitis treating composition.

Another object of the invention is to prepare an aqueous mastitis treating composition which can be infused into the udder of a cow.

A further object is to prepare novel aerosol compositions.

An additional object is to devise an improved method for introducing an antibiotic through a relatively restricted body opening.

In accordance with the invention, there is provided a therapeutic composition effective in the treatment of mastitis of milk animals, by instillation into the animal mammary gland, the composition comprising an aerosol having an aqueous solution of streptomycin or dihydrostreptomycin, neomycin and polymyxin in a nontoxic pressure generating propellant.

Other forms of polymyxin may be used such as polymyxin B. The dihydrostreptomycin, neomycin and polymyxin B can be utilised as either the free base or as the sulfate or other nontoxic salt, e.g., the hydrochloride.

Optionally, sulfa drugs, e.g., sulfmerazine, sulfathiazole and sulfanilamide, tyrothicin and other agents known to be used generally in mastitis preparations may be added to the essential ingredients of the composition according to the invention.

The aqueous base for the medicaments of this invention is intended to include milk itself since milk is essentially water and it can be successfully employed as the vehicle in the aerosol.

The preferred pressure generating propellant in the aerosol is nitrous oxide. Other nontoxic gaseous propellants which can be used include nitrogen, carbon dioxide and the compounds sold under the Registered Trade Mark "Freon" (Dichlorodifluoromethane, trichlorofluoroethane, and trichlorofluoromethane).

It is presently preferred to use a pressure of

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90 lb/sq. in. in the aerosol package although this pressure can be varied as is well known in the aerosol art, e.g., pressures of 25 to 150 psig can be used.

EXAMPLE 1.

In the preferred composition according to the invention there is employed:—

Dihydrostreptomycin (or streptomycin)	50 mg. to 1,000 mg.
Neomycin	50 mg. to 500 mg.
Polymyxin	50,000 units to 1,000,000 units
Water	2.5 cc.

The exact amount of water is not critical but a 2.5 cc. dosage has been found convenient for infusing cows with the composition. Thus there can be used 1 to 30 cc. of water.

EXAMPLE 2.

Dihydrostreptomycin	2,500 mg.
Neomycin	1,000 mg.
Polymyxin B	710,000 units
Water	sufficient to give 25 cc of composition

This composition was placed in a two ounce Wheat Boston round glass bottle and pressured with nitrous oxide to a pressure of 90 psig. The bottle was provided with a metered valve to give 2.5 cc. doses. Thus there was sufficient material in the bottle to supply ten treatments for mastitis. The bottle after filling was provided with an overall coating of a crystalline vinylidene chloride resin which thus sealed all openings and joints. The resin coating was applied by dipping the filled bottle in a solution of the material sold under the Registered Trade Mark "Saran", F-220 (vinylidene chloride-acrylonitrile copolymer) in a mixture of acetone and methyl ethyl ketone and allowing the solvent to dry. It was found that this resin coating greatly improved the shelf life of the mastitis composition.

When the udders of cows having mastitis were infused through the teats with this composition it was found that streptococci infections were 100% reduced and the staphylococci infections reduced over 40% after 120 hours of observation. There is no known mastitis preparation that is 100% effective against staphylococcal mastitis and the results obtained with the compositions of the present invention compare favorably with these obtained with prior art compositions.

TEST 1.

One quarter from a cow was infused through the teat with one dose (2.5 cc.) of the aerosol composition preparted in Example 1 and another quarter of a different cow was infused with three 2.5 cc. doses of the aerosol composition of Example 1. Milk samples from the treated quarters were obtained after 24, 48, and 72 hours following treatment for antibiotic assay by the U.S.P. cylinder-plate method as outlined in Volume XV, pages 848—858 of the U.S. Pharmacopedia. The results of the one and three doses administered were as follows:—

Time after Instillation

	1 Dose				3 Doses			
	5 hrs.	24 hrs.	48 hrs.	72 hrs.	5 hrs.	24 hrs.	48 hrs.	72 hrs.
Dihydrostreptomycin ...	8.5	5.5	0	0	9.1	7.5	5.3	0
Neomycin	6.6	4.39	0	0	7.4	5.6	4.3	0
Polymyxin B	52.9	0	0	0	65.3	45.7	0	0

The results for dihydrostreptomycin and neomycin are expressed in mycrograms/ml. and the results for polymyxin B are expressed in units/ml.

Thus, there was no residue of any antibiotic in the milk 48 hours after the one dose treatment or after 72 hours in the three dose treatment. No untoward signs, such as irritation, were observed in any of the quarters treated with either one or three doses.

TEST 2.

The procedure of test 1 was varied by giving the cows three 2.5 cc. doses 12 hours apart of the aerosol composition of Example 1. The first sample was taken 24 hours after the last dose was given. A total of 11 quarters on 6 different cows were used in this test. No dihydrostreptomycin or polymyxin was detected in the milk after 48 hours. Neomycin was detected in 2 quarters at the end of 48 hours, but at the end of 72 hours even the neomycin was no longer detected.

No residue of antibiotic was observed fater 48 hours in any of 7 quarters of 3 cows given a single 2.5 cc. dose of the aerosol antiobiotic composition of Example 1.

TEST 3.

A total of 164 quarters of 41 milk cows from 3 herds with a history of mastitis were examined clinically and the leucocyte content and bacterial content of milk samples

were determined. Forty quarters was mastitic. At 12 hour intervals 29 of these mastitic quarters were given two 2.5 cc. doses of the aerosol composition of Example 1.

Eleven quarters were infused with one 2.5 cc. dose. Eight of the quarters previously given one dose were infused with three 2.5 cc. doses at 12 hour intervals 17 days after the initial treatment. Samples of the milk from all treated quarters were examined for leucocyte and bacterial content at 0, 24, 48, 72 and 120 hours post treatment, and at 17 days on the 11 quarters given one injection.

The results of this experiment were as follows:

1. At the 120 hour observation 93.1% of the streptococcus infections and 42.7% of the staphylococcus infections were eliminated by the various treatments.

2. The use of one 2.5 cc. dose eliminated 100% of the strepococci and 20% of the staphylococci in 11 quarters for the 17 days observed.

3. 120 hours following the last treatment milk samples from 29 quarters infused with two 2.5 cc. doses at 12 hour intervals showed a reduction of 88.2% streptococci and 41.6% of the staphylococci.

4. 120 hours following the last of 3 treatments with a 2.5 cc. dose there was a reduction by 25% in the staphylococcus infections in 8 quarters that did not respond to the one dose treatment given 17 days previously, making a total of 40% elimination of staphylococci and a 100% elimination of streptococci when the one dose recoveries are included.

5. The aerosol antibiotic composition was nontoxic and there were no symptoms or irritation. There was no flinching or kicking or other indications that cows objected to the udder infusion of the preparation.

Example 3.

In order to improve the stability of the aerosol composition, there can be added conventional preservatives. A typical formulation utilizing preservatives is:

Dihydrostreptomycin	2,500	mg.
Neomycin	1,000	mg.
Polymyxin B	710,000	units
Methyl paraben	50	mg.
Propyl paraben	12.5	mg.
Distilled water	q.s. to make 25	cc.

The above formula was packaged in the 10 plastic coated bottles described in Example 1 to form 10 units dosages with sufficient nitrous oxide at 90 psig. to propel approximately 2.5 cc. of finished product through the valve. This composition produced the same results as in Tests 1—3.

Example 4.

This illustrates the preparation of a commerical batch sufficient for 10,000 bottles (100,000 doses).

Dihydrostreptomycin sulfate powder in an amount equivalent to 25 kilograms of the free base, neomycin sulfate powder in an amount equivalent to 10 kilograms of the free base, polymyxin B sulfate powder in an amount equivalent to 7.1 billion units, 500 grams of methyl paraben and 125 grams of propyl paraben were thoroughly mixed. Then water was added in an amount sufficient to give a total volume of 250 litres. The solution was warmed to 50° C. and held for 10 to 15 minutes. 29 grams of this mixture were then poured into each 2 oz. Wheaten Boston round plastic-coated bottle as in Example 1. A metered Risdon valve (2.5 cc.) was attached to each bottle and nitrous oxide loaded through the valve until an equilibrium pressure of 90 psig. was attained in the bottle.

The final composition was stable for at least 51 days at 45° C. and for at least 87 days at room temperature.

While the use of saran coated bottles is preferred, metal cans and other conventional aerosol containers can be employed.

Disposable plastic teat tubes were employed with the aerosol container to infuse the cows in the examples, although other conventional test tubes can be employed.

The use of the aerosol composition as a therapeutic preparation for the treatment of mastitis has numerous advantages over prior art procedures.

Thus, there is greater ease of application of the therapeutic preparation in introducing it into the udder of the cow. Additionally, there is an efficient measured dose, permitting the faster treatment of the animal.

The propellant was observed to be a nonrefrigerant with no irritating effect in cattle. The aerosol compositions also are suitable for infusing the mammary glands of other mammals, e.g., goats and sheep, by introduction of the antibiotic through the teat.

The use of the overlay of nitrous oxide or other inert gas increases the stability of the antibiotics beyond that normally obtained in mastitis preparations.

Furthermore, there can be used a reduced dosage of a liquid preparation as compared with the prior art.

There is a beneficial increase of phagocytes

and leucocytes following treatment which aids in rapid and natural healing. As a result, there is a more rapid return of the milk to its normal physical and chemical state. The leucocyte and phagocyte stimulation, however, is not sufficient to be an acute inflammatory reaction.

The infusion of nitrous oxide or other inert gas changes the oxygen tension of the udder to such a state that organisms requiring a free oxygen tension are inhibited greatly in function and reproduction.

WHAT WE CLAIM IS:—

1. A therapeutic composition effective in the treatment of mastitis of milk animals, by instillation into the animal mammary gland, the composition comprising an aerosol having an aqueous solution of streptomycin or dihydrostreptomycin, neomycin and polymyxin in a nontoxic pressure generating propellant.

2. A composition according to Claim 1, wherein the propellant is pressured at between 25 and 150 psig and preferably at 90 psig.

3. A composition according to Claim 1 or 2, wherein the propellant is nitrous oxide.

4. A composition according to any one of Claims 1 to 3, having 50 mg. to 1,000 mg. dihydrostreptomycin or streptomycin, 50 mg. to 500 mg. neomycin and 50,000 units to 1,000,000 units of polymyxin per dosage unit.

5. A composition according to Claim 4, wherein said dosage unit includes 1 to 30 c.c., and preferably 2.5 c.c., of water.

6. A composition according to any one of Claims 1 to 3, having 2,500 mg. dihydrostreptomycin, 1,000 mg. of neomycin, 710,000 units of polymyxin B and 25 c.c of water, to form 10 dosage units.

7. A composition according to Claim 8, including as preservative 50 mg. methyl paraben and 12.5 mg propyl paraben.

8. A process for making a therapeutic composition effective in the treatment of mastitis of milk animals by instillation into the animal mammary gland, which comprises the steps of mixing together streptomycin or dihydrostreptomycin, neomycin, polymyxin and water, and pressurising the solution in an aerosol having a nontoxic pressure generating propellant.

9. A process according to Claim 8 wherein the propellant is pressured at between 25 to 150 psig. and preferably at 90 psig.

10. A process according to Claim 8 or 9, wherein the propellant is nitrous oxide.

11. A process according to any one of Claims 8 to 10 wherein there is mixed together 50 mg. to 1,000 mg. dihydrostreptomycin or streptomycin, 50 mg. to 500 mg. neomycin, and 50,000 units to 1,000,000 units of polymyxin to form one dosage unit.

12. A process according to Claim 11 in which the dosage unit includes 1 to 30 c.c., and preferably 2.5 c.c., of water.

13. A process according to any one of Claims 8 to 10, mixing together 2,500 mg. dihydrostreptomycin, 1,000 mg. of neomycin, 710,000 units of polymyxin B and 25 c.c. of water, to form 10 dosage units.

14. A process according to Claim 13 including the additional step of adding as preservative 50 mg. methyl paraben and 12.5 mg. propyl paraben.

15. A process for the treatment of mastitis of milk animals by infusing into the animal's udder with a pressure substantially greater than atmospheric a composition comprising an aqueous solution of streptomycin or dihydrostreptomycin, neomycin and polymyxin in a nontoxic pressure generating propellant.

16. A process according to Claim 15, wherein the propellant is pressured at between 25 to 150 psig. and preferably at 90 psig.

17. A process according to Claim 15 or 16, wherein the propellant is nitrous oxide.

18. A process according to any one of Claims 15 to 17, wherein the mixture has 50 mg. to 1,000 mg. dihydrostreptomycin or streptomycin, 50 mg. to 500 mg. neomycin, and 50,000 units to 1,000,000 units of polymyxin per dosage unit.

19. A process according to Claim 18 wherein said dosage includes 1 to 30 c.c., and preferably 2.5 c.c., of water.

20. A process according to any one of Claims 15 to 17, wherein the mixture has 2,500 mg. dihydrostreptomycin, 1,000 mg. of neomycin, 710,000 units of polymyxin B and 2.5 c.c. of water, to form 10 dosage units.

21. A process according to Claim 20, wherein the mixture includes as preservative 50 mg. methyl paraben and 12.5 mg. propyl paraben.

22. A therapeutic composition according to Claim 1 substantially as herein described.

23. A process for the treatment of mastitis of milk animals substantially as described.

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